ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

Comparison of Intake and Systemic Relative Effect Potencies of Dioxin-like Compounds in Female Mice after a Single Oral Dose

Karin I. van Ede, Patrik L. Andersson, Konrad PJ Gaisch, Martin van den Berg and Majorie BM van Duursen

http://dx.doi.org/10.1289/ehp.1206336

Online 03 May 2013

NIH National Institute of Environmental Health Sciences

Comparison of Intake and Systemic Relative Effect Potencies of Dioxin-like Compounds in Female Mice after a Single Oral Dose

Karin I. van Ede[1], Patrik L. Andersson[2], Konrad PJ Gaisch[1], Martin van den Berg[1], and Majorie BM van Duursen[1]

[1]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands

[2]Department of Chemistry, Umeå University, Umeå, Sweden

Corresponding author:

Karin van Ede

Institute for Risk Assessment Sciences (IRAS)

Utrecht University

P.O. Box 80177

NL-3508 TD Utrecht, the Netherlands

Phone: +31 30 253 3570

e-mail: k.i.vanede@uu.nl

Short running title: Intake and systemic REPs of DLCs in C57Bl/6 mice

Key words: dibenzofurans, dioxins, PCBs, systemic REPs, TEF-concept

Acknowledgements:

This work was financially supported by the European Union Seventh Framework Project SYSTEQ under grant agreement number FP7-ENV-226694. The compounds were purchased by The Dow Chemical Company (Michigan, USA) and donated to the SYSTEQ consortium. The authors thank Helma Avezaat, Sabine Versteeg, Anja van der Sar, Ron Timmermans, Sandra

Nijmeijer, Esmée Janssen, Wouter de Wilde, and Sture Bergek for excellent technical assistance. The authors gratefully thank Dr. Lesa Aylward for all the helpful discussions.

Competing interest declaration:

The purchase of the compounds was financially made possible by The Dow Chemical Company. However, the authors' freedom to design, conduct, interpret, and publish research is not compromised by The Dow Chemical Company as a condition of review and publication.

Abbreviations:

AhR – Aryl hydrocarbon receptor

ANOVA – Analysis of variance

BMR – Benchmark response

CYP – Cytochrome P450

EROD - Ethoxyresorufin-O-deethylase

PBL – Peripheral blood lymphocytes

PCB – Polychlorinated biphenyls

PCB-126 – 3,3',4,4',5-pentachlorobiphenyl

PCB-118 – 2,3',4,4',5-pentachlorobiphenyl

PCB-156 – 2,3,3',4,4',5-hexachlorobiphenyl

PCB-153 – 2,2',4,4',5,5'-hexachlorobiphenyl

PCDD – Polychlorinated dioxin

PCDF – Polychlorinated furan

PeCDD – 1,2,3,7,8-pentachlorodibenzodioxin

4-PeCDF – 2,3,4,7,8,-pentachlorodibenzofuran

REP – Relative effect potency

RfD – Reference dose

TCDD – 2,3,7,8-tetrachlorodibenzodioxin

TDI – Tolerable daily intake

TEF – Toxic equivalency factor

Abstract

Background: Risk assessment for mixtures of chlorinated dioxins (PCDDs), furans (PCDFs) and biphenyls (PCBs) is performed using the toxic equivalency factor (TEF) approach. These TEF values are mainly derived from relative effect potencies (REPs) linking an administered dose to an *in vivo* toxic or biologic effect, resulting in 'intake' TEFs. At present, there is insufficient data available to conclude that intake TEFs are also applicable for systemic concentrations e.g. blood and tissues.

Objective: Compare intake and systemic REPs for 1,2,3,7,8-PeCDD (PeCDD), 2,3,4,7,8-PeCDF (4-PeCDF), 3,3',4,4',5-pentachlorobiphenyl (PCB-126), 2,3',4,4',5-pentachlorobiphenyl (PCB-118) and 2,3,3',4,4',5-hexachlorobiphenyl (PCB-156) in female C57Bl/6 mice three days after a single oral dose.

Method: We calculated intake REPs and systemic REPs based on administered dose, liver, adipose or plasma concentrations relative to TCDD. Hepatic cytochrome P450 1A1 associated ethoxyresorufin-*O*-deethylase (EROD) activity and gene expression of *Cyp1a1*, *1a2* and *1b1* in the liver and peripheral blood lymphocytes (PBLs) were used as biological endpoints.

Results: There is up to one order of magnitude difference between intake REPs and systemic REPs. Two different patterns can be discerned. Based on plasma or adipose levels, systemic REPs are higher for PeCDD, 4-PeCDF and PCB-126, and lower for the mono-*ortho* PCBs 118 and 156 compared to intake REPs.

Conclusions: Based on these mouse data, the comparison between intake REPs and systemic REPs reveals significant congener-specific differences that warrants the development of systemic TEFs to calculate TEQs in blood and body tissues.

Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are persistent and widespread contaminants. In total 419 possible congeners exist of which 7 PCDDs, 10 PCDFs and 12 non-*ortho* and mono-*ortho* PCBs are classified to cause dioxin-like effects. It has been well established that most, if not all, toxic effects of 'dioxin-like' compounds (DLCs) are mediated via the arylhydrocarbon receptor (AHR), causing among others endocrine, developmental, immune and carcinogenic effects (Birnbaum 1994; Birnbaum and Tuomisto 2000; Safe 1990; White and Birnbaum 2009). Humans are exposed to a complex mixture of these DLCs mainly through their diet, with food from animal origin being the most important source. Although exposure has significantly decreased during the last decades (De Mul et al. 2008; Fürst 2006), current human exposure is still above the tolerable daily intake (TDI) or reference dose (RfD) levels for parts of the population in some countries. (Bilau et al. 2008; De Mul et al. 2008; Llobet et al. 2008; Loutfy et al. 2006; Tard et al. 2007). Therefore, improvement of the risk assessment process for this class of compounds remains important and societally relevant.

Currently, risk assessment of DLCs is based on the toxic equivalency factor (TEF) approach (Safe 1990; Safe 1994) endorsed by the World Health Organization (WHO) (Van den Berg et al. 1998; Van den Berg et al. 2006). Each congener-specific TEF is derived from multiple relative effect potencies (REPs) determined from a range of AhR-specific endpoints (e.g. CYP1A1 activity). The toxic or biological potency of a congener is compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). A shortcoming in the present TEF concept originates from the fact that these are mainly established from *in vivo* endpoints linking administered dose levels to toxic or biological effects, resulting in 'intake' TEFs. Consequently, these ^{intake}TEFs are only

applicable for situations in which oral ingestion, like food intake or consumption of breast milk, is known. However, oral ingestion data for humans is often lacking or difficult to establish and blood or adipose tissue levels are frequently used to quantify the relative exposure of humans. Subsequently, regulatory authorities commonly calculate risks based on blood or adipose tissue levels with these ^{intake}TEFs. Unfortunately, even for the most relevant DLCs, sufficient experimental validation is missing that could either reject or accept this application of ^{intake}TEFs for blood or tissue levels. From literature there is limited evidence suggesting that the use of ^{intake}TEFs instead of ^{systemic}TEFs may lead to inaccurate interpretation of the risk due to congener specific toxicokinetic differences (Chen et al. 2001; Devito et al. 1998; Hamm et al. 2003). Properties like absorption, distribution, metabolism, and excretion can clearly contribute to the potency of a congener (Budinsky et al. 2006; Devito and Birnbaum 1995; DeVito et al. 1997; DeVito et al. 2000) and may be misinterpreted when relying solely on ^{intake}TEFs. At the latest 2005 WHO expert meeting, where the TEFs were (re-)evaluated, it was concluded that there was insufficient data available to develop ^{systemic}TEFs, which was considered a major gap in the risk assessment process for DLCs (Van den Berg et al. 2006). To fill this data gap, the EU-project SYSTEQ was initiated with its main objectives to establish *in vivo* ^{systemic}REPs in mouse and rat, with special focus on effects in peripheral blood lymphocytes (PBLs) as potential biomarkers of exposure.

Our present study compares ^{intake}REPs and ^{systemic}REPs in female C57Bl/6 mice based on the administered dose, liver, adipose or plasma concentrations. Experiments were performed with TCDD, PeCDD, 4-PeCDF, PCB-126, PCB-118 and PCB-156, presenting approximately 90% of the dioxin-like activity in the human food chain (Liem et al. 2000), and the non dioxin-like PCB-153. Three days after exposure, we calculated ^{intake}REPs and ^{systemic}REPs for hepatic cytochrome

P450 1A1 (CYP1A1) associated ethoxyresorufin-*O*-deethylase (EROD) activity and *Cyp1a1*, *1a2*, *1b1* gene expression in the liver and PBLs.

Materials and methods

Chemicals

2,3,7,8-tetrachlorodibenzodioxin (TCDD), 1,2,3,7,8-pentachlorodibenzodioxin (PeCDD), 2,3,4,7,8,-pentachlorodibenzofuran (4-PeCDF), and 3,3',4,4',5-pentachlorobiphenyl (PCB-126) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). After dissolving in corn oil (St. Lawrence, USA), concentrations were checked and confirmed by Wellington Laboratories Inc. 2,3',4,4',5-pentachlorobiphenyl (PCB-118), 2,3,3',4,4',5-hexachlorobiphenyl (PCB-156) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) were purchased from Cerilliant Corp. (Round Rock, TX, USA). These PCBs and corn oil (Sigma-Aldrich, Stockholm, Sweden) were purity checked and, when necessary, purified at the Department of Chemistry, Umeå University, Umeå, Sweden. Final TEQ contributions of impurities were 6.6 (PCB-118), 36 (PCB-156) and 0.41 (PCB-153) ng TEQ/g. These levels were considered to have no influence on the final outcome of our results. Further dilutions of the congeners in corn oil (Sigma-Aldrich, Stockholm, Sweden) were prepared at the Institute for Risk Assessment Sciences (IRAS, Utrecht University), The Netherlands.

Animals

Eight-week old female C57Bl/6 mice (Harlan laboratories, Venray, The Netherlands) were randomly assigned to treatment groups (6 animals / group) and allowed to acclimate for 1.5 weeks. The animals were housed in groups in standard cages and conditions (temperature 23 ±

2°C, 50% to 60% relative humidity, 12-h dark and light cycle) with free access to food and water. Mice received a single dose by oral gavage at a dosing volume of 10 ml/kg bw. Depending on the congener, five different dosages were administered ranging from 0.5 µg/kg bw (TCDD) to 500 mg/kg bw (PCB153). For detailed information see Supplemental Material; Table S1. Animals were euthanized at day 3 after dosing by CO_2/O_2. Blood was obtained from the abdominal aorta directly after sacrifice. The liver, thymus and adipose tissue were removed, weighed (liver and thymus), snap frozen and stored until use at -80°C. All animal treatments were performed with permission of the Animal Ethical Committee and performed according to Dutch law on Animal Experiments (http://wetten.overheid.nl/BWBR0003081). The animals were treated humanely and with regard for alleviation of suffering.

Compound analysis

Adipose and liver tissues samples were homogenized in Na_2SO_4 followed by extraction and clean-up in one step, including elution with 200 ml 1:1 hexane:dichloromethane on an open column packed with 40% w/w H_2SO_4 impregnated silica and KOH-silica. Blood plasma samples were extracted on an open column using Chem-Elut and NaCl eluted with 75 ml 3:2 hexane:2-propanol. Clean-up was performed using a miniaturized silica column (as described above) eluted using 30 ml hexane. The samples typically contained high levels of the analytes and thus only a small fraction was evaporated and analysed. Prior to evaporation, a fraction of the samples was spiked with ^{13}C-labeled standards. Potential loss of analytes during extraction and clean-up were checked by re-extracting the samples using identical protocol as for the samples. This procedure indicated that only minor losses have occurred during this first step that most likely does not significantly contribute to the measured outcomes. Tetradecane was added as a keeper prior to evaporation. Sample analysis followed the US EPA Method 1613

(http://water.epa.gov/scitech/methods/cwa/organics/dioxins/index.cfm) using single ion monitoring mode on a Hewlett Packard 5890 GC coupled to a Fisons Instruments VG Autospec HRMS. Compounds were separated on a 60m×0.25mm DB5-MS column (0.25µm, J&W Scientific, Folsom, CA, USA). The HRMS was operated with electron impact ionization with electron energy of 35 eV and an ion source temperature of 250 °C. To reduce the number of analyses, samples were pooled prior to clean-up. To retain unique individual results, liver, adipose and plasma samples were not pooled within the same treatment group of one congener, but between similar exposure levels of TCDD, PeCDD, 4-PeCDF and PCB126 or PCB-118, PCB-156 and PCB-153. This method could be applied because full congener specific separation could be achieved on the HRGC-HRMS. For lipid determination, samples were evaporated to dryness after the extraction step and the amount of lipids was determined gravimetrically. Concentrations were calculated based on lipid and wet weight. The analysis of PCB 118 dose 5000 µg/kg bw failed in the sampling procedure, analysis for this group could not be completed.

Plasma and peripheral blood lymphocyte (PBL) isolation

Blood from two mice was pooled (total volume ± 1.4 ml) after which plasma and PBLs were isolated using Ficoll Paque gradient (GE Healthcare Europe, Diegem, Belgium). Plasma samples were stored directly at -80°C until compound analysis. Isolated lymphocytes were lysed with RLT buffer (QIAGEN, Venlo, the Netherlands) as described in the QIAGEN RNAeasy kit protocol and stored until use at -80°C.

EROD activity

Hepatic CYP1A1 activity was determined using ethoxyresorufin-*O*-deethylase (EROD) activity in hepatic microsomal fractions according to Schulz *et al*. (Schulz et al. 2012).

RNA isolation and quantitative real-time PCR

Total RNA was isolated from liver and PBLs using a QIAGEN RNeasy kit (QIAGEN, Venlo, The Netherlands). Purity and concentration of the isolated RNA was determined by measuring the absorbance ratio at 260/280nm and 230/260nm with a Nanodrop 2000 spectrophotometer (Thermo Scientific). RNA was reverse transcribed to complementary DNA (cDNA) using the iScript cDNA synthesis Kit (Bio-Rad, Veenendaal, the Netherlands). Quantitative real-time PCR (RT-PCR) analyses were performed using the iQ Real-Time PCR Detection System with SYBR green (Bio-rad, Veenendaal, the Netherlands). Amplification reactions were set up with 15 µl mastermix containing 12.5 µl iQ SYBR Green Supermix from Bio-rad, 0.5 µl dH_2O, 1 µl (10 µM) forward primer [FW], 1 µl (10 µM) reserve primer [RV]) and 10 µl first strand cDNA (10X diluted). Primer sequences were as follows: *Cyp1a1*, FW-5'-GGTTAACCATGACCGGGAACT-3' and RV-5'-TGCCCAAACCAAAGAGAGTGA-3' (Schulz et al. 2012) *Cyp1a2*, FW-5'-ACATTCCCAAGGAGCGCTGTATCT-3' and RV-5'-GTCGATGGCCGAGTTGTTATTGGT-3' (Flaveny et al. 2010) *Cyp1b1*, FW-5'-GTGGCTGCTCATCCTCTTTACC-3' and RV-5'-CCCACAACCTGGTCCAACTC-3' (Berge et al. 2004) *β-actine*, FW-5'-ATGCTCCCCGGGCTGTAT-3' and RV-5'-CATAGGAGTCCTTCTGACCCATTC-3' (Schulz et al. 2012). All primers were run through National Center for Biotechnology Information (NCBI) Primer-BLAST database to confirm specificity and validated for optimal annealing temperature (60°C for all primers) and efficiency. The efficiency of all primer pairs was 98% - 102% (tested at 60°C). The following program was used for denaturation and amplification of the cDNA: 3 min at 95°C, followed by 40 cycles of 15s at 95°C and 45s at 60°C. Gene expression for each sample was expressed as threshold cycle

(C_t), normalized to the reference gene β-actine (ΔC_t). Fold induction was calculated between the treated and control group.

Data analysis

Concentration-response curves were obtained using a sigmoidal dose-response nonlinear regression curve fit with variable slope (GraphPad Prism 5.04, GraphPad Software Inc., San Diego, CA) [1].

$$y = E_0 + \left(\frac{(E_{max} * X^n)}{(b^n + X^n)}\right) \qquad [1]$$

In this Hill equation, y is the dependent variable (EROD activity or fold induction of mRNA levels) and x the independent variable (administered or systemic dose). E_0 is the estimated background response level, E_{max} the maximum response, b is the estimated EC_{50} and n is the shaping parameter of the Hill curve.

The potency of a congener was calculated relatively to TCDD using the dose or concentration needed for a congener to reach 20% response of TCDD (BMR_{20TCDD}). Using the congener specific BMR_{20TCDD} concentration, relative effect potencies (REPs) were calculated relatively to TCDD [2].

$$REP\ congener\ X = \frac{BMR_{20TCDD}\ of\ TCDD}{BMR_{20TCDD}\ of\ congener\ X} \qquad [2]$$

Statistical analysis

Statistical significant differences of the means and variances were determined using analysis of variance (one-way ANOVA) test followed by a Tukey-Kramer multiple comparisons test.

Differences were considered statistically significant if $p < 0.05$. Statistical calculations were performed using GraphPad 6.01 (GraphPad Software Inc., San Diego, CA).

Results

Effect on body and organ weight

To evaluate the possible toxic effects of the congeners tested, we examined body and organ weights. Compared to vehicle control-treated mice, there were no changes in body weight in treated mice upon sacrifice. Relative thymus weights showed a decreasing trend for all compounds, except PCB-126. However, this decrease was only statistically significantly different from the vehicle control-treated mice for TCDD (≥ 2.5 μg/kg bw), PeCDD (0.5, 10 and 100 μg/kg bw) and PCB-153 (500 mg/kg bw). Furthermore, a dose-dependent increasing trend in liver weight was seen for all compounds. This increase in liver weight was significantly different from vehicle control treated mice at doses similar or higher than 10, 100, 100, 1000, 150000, 50000 and 500000 μg/kg bw, for TCDD, PeCDD, 4-PeCDF, PCB-126, PCB-118, PCB-156 and PCB-153, respectively. Also, the hepatic lipid content showed a dose-dependent increasing trend compared to vehicle control-treated mice for all compounds, except PCB-153. No statistically significant changes in spleen weight were observed for any of the compounds tested. More detailed information is provided in Supplemental Material; Table S2.

Distribution of the compounds

To calculate ^{systemic}REPs, we analyzed liver, adipose and plasma concentrations (See Supplemental Material; Table S3). Within the three days period between dosage and sacrifice, concentrations of all congeners increased linearly with the administered dose (see Figure 1).

This observation indicates an absence of auto-induction of metabolism for the different dose levels within this time period.

On a wet weight basis (ng/g tissue), concentrations in the liver were higher than in the adipose tissue for TCDD, PeCDD, 4-PeCDF and PCB-126 (See Supplemental Material; Table S3). In contrast, liver concentrations were lower compared to adipose tissue for the mono-*ortho* PCBs 156 and 118, and the non dioxin-like PCB 153. These differences became even more pronounced when expressing the concentrations as % dose/g tissue. Thus, it can be concluded that the more potent DLCs had a higher liver affinity than the less potent PCBs 118 and 156. Therefore, we determined the ratio between liver and adipose tissue to study congener-specific hepatic sequestration. Earlier, it was suggested that a liver:adipose ratio greater than 0.3 reflects a congener-specific hepatic sequestration (Diliberto et al. 1997). In our study, this was found for TCDD, PeCDD, 4-PeCDF and PCB-126, while in contrast, liver:adipose ratios less than 0.3 were observed for the PCBs 118, 156 and 153 (Table 1). Also, hepatic sequestration was dose-dependent for TCDD and PCB126, as shown by increasing liver:adipose ratios at higher dose levels. This was not observed for PeCDD and 4-PeCDF.

Dose-response curves

With the available tissue and plasma concentrations, we determined dose-response relationships of hepatic EROD activity and gene expressions of *Cyp1a1*, *1b1* and *1a2* in liver and PBLs (See Supplemental Material; Figure S1). All compounds, except PCB-153, caused a statistically significant, dose-dependent increase in hepatic EROD activity as well as *Cyp1a1* and *1a2* mRNA levels. Hepatic *Cyp1b1* mRNA expression was dose-dependently increased by TCDD, PCDD, 4-PeCDF and PCB-156. A dose-dependent trend was seen for PCB-118, however, the maximum

induction for PCB-118 was below 0.3% of the maximal response of TCDD. PCB-126 did not induce *Cyp1b1* mRNA levels in the liver. In PBLs, *Cyp1a1* mRNA levels were dose-dependently induced by all compounds, except PCB-118 and PCB-153. *Cyp1b1* mRNA was statically significant and dose-dependently induced by TCDD, PeCDD and 4-PeCDF. PCB-126 induced *Cyp1b1* mRNA only at the highest dose tested with 3,5% of the maximal induction by TCDD. PCB-118, PCB-156 and PCB-153 did not induce *Cyp1b1* mRNA levels in PBLs. *Cyp1a2* mRNA was not expressed in PBLs.

Interestingly, for all DLCs, a maximum induction (Y_{max}) was only reached for hepatic EROD activity and not for *Cyp1a1*, *1b1* and *1a2* mRNA in the liver and PBLs, even at the highest doses tested. Furthermore, differences in curve Hill slopes between congeners were observed for all endpoints tested (Supplemental Material; Figure S1). Furthermore, in Supplemental Material; Figure S2, dose response curves of *Cyp1a1* mRNA in liver and PBLs based on administered dose, liver or plasma concentration are shown. Congener-specific differences in Y_{max} and Hill slopes can add a significant uncertainty in calculating EC_{50} values that generally form the basis of REP determination. To reduce this uncertainty, it was decided to focus on the lower part of the dose-response curves with a benchmark response of 20% of the Y_{max} of TCDD (BMR_{20TCDD}) as comparative endpoint (Supplemental Material; Figure S1 and S2).

BMR_{20TCDD} concentrations and Relative Effect Potencies (REPs)

BMR_{20TCDD} values for hepatic endpoints were calculated based on administered dose, hepatic, adipose or plasma concentration, whereas BMR_{20TCDD} for PBL endpoints were only calculated using the administered dose or plasma concentration. The administered dose or systemic levels needed for a congener to reach a 20% effect of TCDD varied strongly between endpoints, but

also between the liver and PBLs (Table 2). In PBLs, a higher concentration was usually needed to reach a BMR_{20TCDD} compared to liver for the same endpoint. In the liver, EROD activity was the most sensitive biomarker for TCDD, PeCDD, 4-PeCDF and PCB-126 exposure, followed by *Cyp1a1* and *Cyp1a2* mRNA induction. In contrast, hepatic *Cyp1a2* mRNA induction appeared to be the most sensitive biomarker for PCB-118 and 156, followed by EROD activity and *Cyp1a1* gene expression. In PBLs, the BMR_{20TCDD} of *Cyp1a1* and *Cyp1b1* were similar for TCDD. In contrast, for PCDD and 4-PeCDF BMR_{20TCDD} for *Cyp1b1* were at least 2-fold higher compared to *Cyp1a1* gene expression. In Figure 2, an overview is given for the REP differences based on liver, adipose or plasma concentrations. Here, it is important to note that a BMR_{20TCDD} was not reached for all congeners and endpoints studied. These data were excluded from the REP calculations.

For comparison of congener specific REPs across exposure matrices (intake, liver, adipose, or plasma), the ^{intake}REP was set to 1 and deviations were calculated for various ^{systemic}REPs with the same endpoint (Figure 2). Two different types of deviations from ^{intake}REPs can be observed for ^{systemic}REPs in this study. Based on liver concentrations (wet or lipid weight) ^{systemic}REPs of PeCDD, 4-PCDF and PCB-126 are approximately one third of the ^{intake}REP at most. In contrast, ^{systemic}REPs of PCBs 118 and 156 are up one order of magnitude higher than the ^{intake}REPs. ^{systemic}REPs for hepatic effects of PeCDD, 4-PeCDF and PCB126 were also calculated using adipose tissue and plasma concentrations. In this case, ^{systemic}REPs are up to one order of magnitude higher compared to ^{intake}REPs, depending on the endpoint studied. The opposite was again found for the ^{systemic}REPs of PCB-118 and PCB-156 that are at most one third of the ^{intake}REP. ^{systemic}REPs for effects in PBLs based on plasma concentrations reveal similar

deviations from ^{intake}REPs, as observed for ^{systemic}REPs of hepatic endpoints based on plasma concentration.

In summary, two different types of deviations from ^{intake}REPs are found for ^{systemic}REPs, differentiating the more potent AhR agonists PeCDD, 4-PeCDF and PCB-126 from the less potent mono-*ortho* PCBs 118 and 156. In both groups, ^{systemic}REPs can differ as much as one order of magnitude from the ^{intake}REPs (see Figure 2).

Discussion

The TEF approach is the most commonly used method around the world to assess the risk of complex mixtures of dioxins and dioxin-like compounds. Current TEF values are mainly derived from a range of ^{intake}REPs preferably from (sub)chronic *in vivo* studies. These ^{intake}REPs link the administered dose to a toxic or biological effect, subsequently leading to the derivation of ^{intake}TEFs (Van den Berg et al. 1998; Van den Berg et al. 2006).

At present, there is insufficient data, to establish whether or not ^{intake}TEFs are valid for risk assessment based on plasma or adipose tissue concentrations. So far, the limited experimental evidence available suggests that ^{systemic}REPs of DLCs may differ from ^{intake}REPs (Budinsky et al. 2006; DeVito et al. 1997; DeVito et al. 2000). This discrepancy originates most likely from toxicokinetic differences between various DLCs. Several studies have shown that many DLCs bind strongly to CYP1A2 protein and as a result strongly sequester in the rodent liver (Devito et al. 1998; Diliberto et al. 1995; Diliberto et al. 1997; Diliberto et al. 1999). This binding affinity towards CYP1A2 influences the hepatic, plasma and adipose tissue disposition of DLCs. This was confirmed by using CYP1A2 knockout mice in which the liver:adipose ratio decreased to

below 0.3 for TCDD and 4-PeCDF, which is indicative of no hepatic sequestration (Diliberto et al. 1997). These ratios are significantly lower than those observed in this study for both congeners (see Table 1). It is worth nothing that, the dose-dependency and hepatic sequestration observed in our single dose, 3-day study are similar to those observed in a multiple dose, subchronic 13 weeks study with female B6C3F1 mice for all tested compounds, except for 4-PeCDF at the two highest concentrations tested (Devito et al. 1998). Also, the responding TCDD EC_{50} systemic liver concentrations for hepatic EROD activity were similar. Comparable findings can also be expected for the other DLCs tested, since metabolism and elimination for these compounds are very similar. In this view, it may be expected that ^{intake}REPs and ^{systemic}REPs do not deviate over time, even though a steady state is not yet reached. In our study, ^{intake}REPs and ^{systemic}REPs for *Cyp1a1*, *1a2* and *1b1* induction are determined three days after a single oral dose. Previous studies have shown that after 3 days hepatic CYP1A1, 1A2 and 1B1 protein levels are already maximal in rats following a single dose of TCDD (Santostefano et al. 1997). Although induction of CYP1A1, 1A2 and 1B1 enzymes is not a measure of toxicity, this is considered to be the most sensitive biomarker for AHR activation (Abel and Haarmann-Stemmann 2010; Denison and Heath-Pagliuso 1998). Moreover, multiple studies have shown a high correlation in REPs between induction of these enzymes and toxic responses inflicted by DLCs, such as wasting syndrome, thymic atrophy or hepatic porphyrin accumulation (Safe 1990; Van Birgelen et al. 1996).

In line with earlier studies, we observe distinct deviations between ^{intake}REPs and ^{systemic}REPs based on liver, plasma or adipose tissue concentrations (Budinsky et al. 2006; Devito and Birnbaum 1995; DeVito et al. 1997; DeVito et al. 2000). Congener-specific differences are found between the potent PeCDD, 4-PeCDF and PCB-126 versus the less potent mono-*ortho* PCBs 118

and 156 (see Figure 2). Based on the liver:adipose ratios established in our study (see Table 1), it appears that these differences have a toxicokinetic basis, in which hepatic sequestration due to CYP1A2 binding, plays a significant role. Currently, it is unclear whether a CYP1A2-sequestered compound is bioavailable to activate the AhR and cause dioxin-like responses. For this reason, REPs calculated on total hepatic tissue concentration, instead of the "free" available concentrations, may lead to either an over- or under-estimation of the potency of a congener, depending on the relative degree of hepatic sequestration compared to TCDD. The ^{systemic}REPs based on plasma concentrations for *Cyp1a1* and *1b1* gene expression in PBLs and liver show similar deviations from ^{intake}REPs for all DLCs tested. The ^{systemic}REPs are sometimes more than half a log unit different from the ^{intake}REPs, which is more than the assumed uncertainty range applied to the present WHO-TEF values (Van den Berg et al. 2006). To further address this issue, we compared ^{intake}REPs and ^{systemic}REPs from this study with existing WHO-TEFs and the half log uncertainty around that value (Figure 3). Based on this comparison, a number of observations can be made:

- REPs of PeCDD fall mostly within the uncertainty range of the WHO-TEF of 1 with no large difference between ^{systemic}REPs and ^{intake}REPs.
- Based on the intake dose and hepatic concentrations, deviations from the half log unit uncertainty are observed for 4-PeCDF, but ^{systemic}REPs based on plasma concentrations are close to the WHO-TEF of 0.3.
- For PCB-126, ^{intake}REPs and ^{systemic}REPs are up to two orders of magnitude below the WHO-TEF value of 0.1. Of all endpoints studied only *Cyp1a1* mRNA expression in PBLs falls within the half log unit uncertainty.

- REPs based on intake dose and plasma concentrations for mono-*ortho* PCBs 118 and 156 are consistently lower than the WHO-TEFs of 0.00003. In contrast, REPs based on liver effects and concentrations are significantly higher than the WHO-TEF for both PCBs. However, due to differences in Cyp1a2-sequestration between the mono-*ortho* PCBs and the reference compound TCDD, caution should be taken not to over-interpret these liver based ^{systemic}REPs.

Most REPs determined in this study are significantly lower than those established by the WHO (Van den Berg et al. 2006). However, it should be noted that WHO-TEFs were derived from a range of ^{intake}REPs often involving (semi)chronic studies and different species, while our study involves a single dose exposure with relatively acute effects after three days in mice only. The present study did not aim to recalculate or debate the current WHO-TEFs or its methodology. However, the current WHO-TEF concept is based on the assumption that ^{intake}REPs represent ^{systemic}REPs, while a full data set to reject or accept this assumption is lacking. In this study, we compare ^{intake}REPs with ^{systemic}REPs obtained from a mouse model to provide more knowledge about possible deviations between both type of REPs. More data, for example additional *in vivo* rat data and human *in vitro* data from our EU-SYSTEQ project studies, may provide additional information with respect to deviation of the ^{intake}REPs and ^{systemic}REPs from our studies with current WHO-TEF values. In this light, it can then be discussed whether ^{systemic}REPs, would better reflect a risk than "intake" (WHO-)TEFs.

Conclusions

There are significant differences between ^{intake}REPs and ^{systemic}REPs based on administered dose or liver, adipose tissue and plasma concentrations for hepatic EROD activity and *Cyp1a1*, *1a2*

and *1b1* gene expression in the liver and PBLs. To avoid flawed calculations due to e.g. congener-specific hepatic sequestration, selecting blood or adipose tissue as matrix to calculate ^{systemic}REPs may be more appropriate than hepatic levels. The ^{systemic}REPs based on plasma / adipose concentration in our study are sometimes more than half a log unit different from the ^{intake}REPs. This implies that using ^{intake}REPs or ^{intake}TEFs may lead to an underestimation of the risk if these are used to calculate TEQs in blood for PeCDD, 4-PeCDF and PCB-126. In contrast, using ^{intake}REPs or ^{intake}TEFs for the mono-*ortho* PCBs 118 and 156 to calculate blood TEQs in blood may lead to an overestimation of the risk. Overall, based on these mouse data, a comparison between the ^{intake}REPs and ^{systemic}REPs reveals significant congener-specific differences that warrants the development of ^{systemic}TEFs to calculate TEQs in blood and body tissues.

References

Abel J, Haarmann-Stemmann T. 2010. An introduction to the molecular basics of aryl hydrocarbon receptor biology. Biol Chem 391(11):1235-1248

Berge G, Øvrebø S, Eilertsen E, Haugen A, Mollerup S. 2004. Analysis of resveratrol as a lung cancer chemopreventive agent in A/J mice exposed to benzo[a]pyrene. Br J Cancer 91(7):1380-1383

Bilau M, Matthys C, Baeyens W, Bruckers L, Backer GD, Hond ED et al. 2008. Dietary exposure to dioxin-like compounds in three age groups: Results from the flemish environment and health study. Chemosphere 70(4):584-592

Birnbaum LS. 1994. The mechanism of dioxin toxicity: Relationship to risk assessment. Environ Health Perspect 102(SUPPL. 9):157-167.

Birnbaum LS, Tuomisto J. 2000. Non-carcinogenic effects of TCDD in animals. Food Addit Contam 17(4):275-288.

Budinsky RA, Paustenbach D, Fontaine D, Landenberger B, Starr TB. 2006. Recommended relative potency factors for 2,3,4,7,8-pentachlorodibenzofuran: The impact of different dose metrics. Toxicol Sci 91(1):275-285

Chen CY, Hamm JT, Hass JR, Birnbaum LS. 2001. Disposition of polychlorinated dibenzo-p-dioxins, dibenzofurans, and non-ortho polychlorinated biphenyls in pregnant long evans rats and the transfer to offspring. Toxicol Appl Pharmacol 173(2):65-88.

De Mul A, Bakker MI, Zeilmaker MJ, Traag WA, Leeuwen SPJv, Hoogenboom RLAP et al. 2008. Dietary exposure to dioxins and dioxin-like PCBs in the netherlands anno 2004. Regul Toxicol Pharmacol 51(3):278-287

Denison MS, Heath-Pagliuso S. 1998. The ah receptor: A regulator of the biochemical and toxicological actions of structurally diverse chemicals. Bull Environ Contam Toxicol 61(5):557-568.

Devito MJ, Birnbaum LS. 1995. The importance of pharmacokinetics in determining the relative potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran. Toxicol Sci 24(1):145-148

DeVito MJ, Diliberto JJ, Ross DG, Menache MG, Birnbaum LS. 1997. Dose-response relationships for polyhalogenated dioxins and dibenzofurans following subchronic treatment in mice: I. CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin. Toxicol Appl Pharmacol 147(2):267-280

DeVito MJ, Menache MG, Diliberto JJ, Ross DG, Birnbaum LS. 2000. Dose-response relationships for induction of CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin in female mice following subchronic exposure to polychlorinated biphenyls. Toxicol Appl Pharmacol 167(3):157-172.

Devito MJ, Ross DG, Dupuy A.E. J, Ferrario J, McDaniel D, Birnbaum LS. 1998. Dose-response relationships for disposition and hepatic sequestration of polyhalogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls following subchronic treatment in mice. Toxicol Sci 46(2):223-234

Diliberto JJ, Akubue PI, Luebke RW, Birnbaum LS. 1995. Dose-response relationships of tissue distribution and induction of CYP1A1 and CYP1A2 enzymatic activities following acute exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice. Toxicol Appl Pharmacol 130(2):197-208.

Diliberto JJ, Burgin D, Birnbaum LS. 1997. Role of CYP1A2 in hepatic sequestration of dioxin: Studies using CYP1A2 knock-out mice. Biochem Biophys Res Commun 236(2):431-433

Diliberto JJ, Burgin DE, Birnbaum LS. 1999. Effects of CYP1A2 on disposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin, 2,3,4,7,8-pentachlorodibenzofuran, and 2,2',4,4',5,5'-hexachlorobiphenyl in CYP1A2 knockout and parental (C57BL/6N and 129/Sv) strains of mice. Toxicol Appl Pharmacol 159(1):52-64

Flaveny CA, Murray IA, Perdew GH. 2010. Differential gene regulation by the human and mouse aryl hydrocarbon receptor. Toxicol Sci 114(2):217-225

Fürst P. 2006. Dioxins, polychlorinated biphenyls and other organohalogen compounds in human milk. Mol Nutr Food Res 50(10):922-933

Hamm JT, Chen CY, Birnbaum LS. 2003. A mixture of dioxins, furans, and non-ortho PCBs based upon consensus toxic equivalency factors produces dioxin-like reproductive effects. Toxicol Sci 74(1):182-191.

Haws LC, Su SH, Harris M, DeVito MJ, Walker NJ, Farland WH et al. 2006. Development of a refined database of mammalian relative potency estimates for dioxin-like compounds. Toxicol Sci 89(1):4-30.

Liem AKD, Fürst P, Rappe C. 2000. Exposure of populations to dioxins and related compounds. Food Addit Contam 17(4):241-259

Llobet JM, Martí-Cid R, Castell V, Domingo JL. 2008. Significant decreasing trend in human dietary exposure to PCDD/PCDFs and PCBs in catalonia, spain. Toxicol Lett 178(2):117-126

Loutfy N, Fuerhacker M, Tundo P, Raccanelli S, El Dien AG, Ahmed MT. 2006. Dietary intake of dioxins and dioxin-like PCBs, due to the consumption of dairy products, fish/seafood and meat from ismailia city, egypt. Sci Total Environ 370(1):1-8

Safe S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit Rev Toxicol 21(1):51-88

Safe SH. 1994. Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol 24(2):87-149.

Santostefano MJ, Ross DG, Savas U, Jefcoate CR, Birnbaum LS. 1997. Differential time-course and dose-response relationships of TCDD-induced CYP1B1, CYP1A1, and CYP1A2 proteins in rats. Biochem Biophys Res Commun 233(1):20-24.

Schulz VJ, Smit JJ, Huijgen V, Bol-Schoenmakers M, Van Roest M, Kruijssen LJW et al. 2012. Non-dioxin-like AhR ligands in a mouse peanut allergy model. Toxicol Sci 128(1):92-102

Tard A, Gallotti S, Leblanc J-, Volatier J-. 2007. Dioxins, furans and dioxin-like PCBs: Occurrence in food and dietary intake in france. Food Addit Contam 24(9):1007-1017

Van Birgelen APJM, Fase KM, Van der Kolk J, Poiger H, Brouwer A, Seinen W et al. 1996. Synergistic effect 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic porphyrin levels in the rat. Environ Health Perspect 104(5):550-557.

Van den Berg M, Birnbaum L, Bosveld ATC, Brunström B, Cook P, Feeley M et al. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ Health Perspect 106(12):775-792.

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M et al. 2006. The 2005 world health organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93(2):223-241.

White SS, Birnbaum LS. 2009. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27(4):197-211.

Table 1: Liver:adipose concentration ratios.

Congener	Dose μg/kg bw	Ratio[a] liver:adipose
TCDD	0.5	1.8 ± 0.2
	2.5	2.9 ± 0.5[b]
	10	4.2 ± 0.7[b]
PeCDD	0.5	4.4 ± 0.9
	2.5	7.0 ± 1.1[b]
	10	6.7 ± 1.4
4-PeCDF	5	11.5 ± 1.7
	25	13.2 ± 1.5
	100	13.3 ± 2.6
PCB-126	5	3.2 ± 0.3
	25	5.9 ± 0.9[b]
	100	9.1 ± 0.9[b]
PCB-118	15000	0.08 ± 0.01
	50000	0.07 ± 0.02
PCB-156	5000	0.09 ± 0.02
	15000	0.11 ± 0.03
	50000	0.12 ± 0.02
PCB-153	5000	0.08 ± 0.02
	15000	0.11 ± 0.02
	50000	0.08 ± 0.03

[a]Liver and adipose concentrations (in ng/g tissue) were used to calculate congener specific ratios. Data represents the mean ± SD of 6 mice. Statistically significant changes were determined by one-way ANOVA analysis followed by a Tukey's multiple comparisons test.

[b]Significantly different from previous concentration ($p < 0.05$).

Table 2: Mean BMR_{20TCDD} concentrations for TCDD, PeCDD, 4-PeCDF, PCB-126, PCB-118 and PCB-156 and corresponding relative effect potencies (REPs) for various endpoints in liver and peripheral blood lymphocytes

Biomarker	Dose metric	TCDD		PeCDD		4-PeCDF		PCB-126		PCB-118		PCB-156	
		BMR_{20TCDD}	REP	BMR_{20TCDD}	REP	BMR_{20TCDD}	REP	BMR_{20TCDD}	REP	BMR_{20TCDD}	REP	BMR_{20TCDD}	REP
Liver	Adm. dose (µg/kg bw)	0.29	1	0.54	0.5	4.11	0.07	29.3	0.01	55259	0.000005	15664	0.00002
EROD activity	Sys. liver (ng/g liver)	1.61	1	4.85	0.3	32.9	0.05	373	0.004	25441	0.00006	7501	0.0002
	Sys. liver (ng/g lipid)	34.6	1	99.6	0.3	913	0.04	9938	0.003	720241	0.00006	217711	0.0002
	Sys. adipose (ng/g lipid)	1.23	1	1.25	1	3.47	0.4	72.7	0.02	359114	0.000003	82483	0.00001
	Sys. plasma (ng/g lipid)	1.38	1	2.31	0.6	3.50	0.4	72.3	0.02	311118	0.000004	98188	0.00001
Liver	Adm. dose (µg/kg bw)	0.64	1	1.25	0.5	81.3	0.008	558	0.001	139631	0.000005	44305	0.00001
mRNA *Cyp1a1*	Sys. liver (ng/g liver)	4.35	1	12.0	0.4	725	0.006	4299	0.001	62418	0.00007	35669	0.0001
	Sys. liver (ng/g lipid)	77.5	1	216	0.4	13768	0.006	70368	0.001	1693882	0.00005	634215	0.0001
	Sys. adipose (ng/g lipid)	2.50	1	2.36	1	59.8	0.04	315	0.008	ND		180515	0.00001
	Sys. plasma (ng/g lipid)	2.66	1	3.94	0.7	37.6	0.07	0.32	0.008	803766	0.000003	303586	0.000009
Liver	Adm. dose (µg/kg bw)	3.55	1	10.1	0.4	150	0.02	ND		ND		95664	0.00004
mRNA *Cyp1b1*	Sys. liver (ng/g liver)	29.1	1	105	0.3	1577	0.02	ND		ND		72445.7	0.0004
	Sys. liver (ng/g lipid)	391	1	1655	0.2	32921	0.01	ND		ND		1158251	0.0003
	Sys. adipose (ng/g lipid)	10.3	1	19.4	0.5	461	0.02	ND		ND		745126	0.00001
	Sys. plasma (ng/g lipid)	11.6	1	18.5	0.6	44.6	0.3	ND		ND		553459	0.00002
Liver	Adm. dose (µg/kg bw)	0.41	1	0.56	0.7	8.83	0.05	87.4	0.005	15522	0.00003	12085	0.00003
mRNA *Cyp1a2*	Sys. liver (ng/g liver)	2.59	1	4.59	0.6	68.1	0.04	912	0.003	8833	0.0003	4239	0.0006
	Sys. liver (ng/g lipid)	51.1	1	95.3	0.5	1712	0.03	21240	0.002	267405	0.0002	166060	0.0003
	Sys. adipose (ng/g lipid)	1.73	1	1.20	1	6.53	0.3	120	0.01	117517	0.00001	22134	0.00008
	Sys. plasma (ng/g lipid)	1.85	1	2.36	0.8	8.01	0.2	135	0.01	103230	0.00002	60702	0.00003
PBLs	Adm. dose (µg/kg bw)	22.4	1	33.7	0.7	117	0.2	603	0.04	ND		747734	0.00003
mRNA *Cyp1a1*	Sys. plasma (ng/g lipid)	50.6	1	34.0	1.5	40.8	1	847	0.06	ND		2359081	0.00002
PBLs	Adm. dose (µg/kg bw)	20.9	1	51.8	0.4	514	0.04	ND		ND		ND	
mRNA *Cyp1b1*	Sys. plasma (ng/g lipid)	53.5	1	63.8	0.8	212	0.3	ND		ND		ND	
PBLs	Adm. dose (µg/kg bw)	ND		ND		ND		ND		ND		ND	
mRNA *Cyp1a2*	Sys. plasma (ng/g lipid)	ND		ND		ND		ND		ND		ND	

Data are expressed as mean BMR_{20TCDD} derived from dose-response curves of 6 mice. REPs are calculated as described in Materials & Methods.

ND = not determined, because BMR_{20TCDD} was not reached; PBLs = Peripheral blood lymphocytes

Figure legends:

Figure 1. Relation between oral dose and mean systemic concentration in mouse liver (—) or adipose tissue (---) for TCDD, PeCDD, 4-PeCDF, PCB-126, PCB-118, PCB-156 and PCB-153. Systemic concentrations were determined in female C57bl/6 mice, 3 days after administration of a single oral dose. Data points represent the oral dose and the mean tissue concentration ± SD of 6 mice.

Figure 2. Fold change in ^{systemic}REP compared to ^{intake}REP for PeCDD, 4-PeCDF, PCB-126, PCB-118 and PCB-156. Changes in REPs are calculated for hepatic EROD activity (▬) and *Cyp1a1* (▭) , *Cyp1b1* (▭) and *Cyp1a2* (▭) gene expression in liver and PBLs.

ND = not determined.

Figure 3. Relative effect potencies (REPs) determined in this study in relation to the WHO-TEF ± half log uncertainty range. REPs were determined for hepatic EROD activity(☒), hepatic gene expression of *Cyp1a1* (■), *Cyp1b1* (☐), *Cyp1a2* (▯), and gene expression of *Cyp1a1* (●) and *Cyp1b1* (◐) in PBLs of PeCDD, 4-PeCDF, PCB-126 (left graph) and PCB-118 and PCB-156 (right graph). REPs for hepatic endpoints were calculated based on administered dose (Intake), lipid-based liver concentration (Liver) or lipid-based plasma concentration (Plasma), whereas for PBL, REPs were calculated using the administered dose or plasma concentration. The black line represents the mean of the REPs. The black dotted line together with its grey area represents the WHO-TEF ± half log uncertainty range.

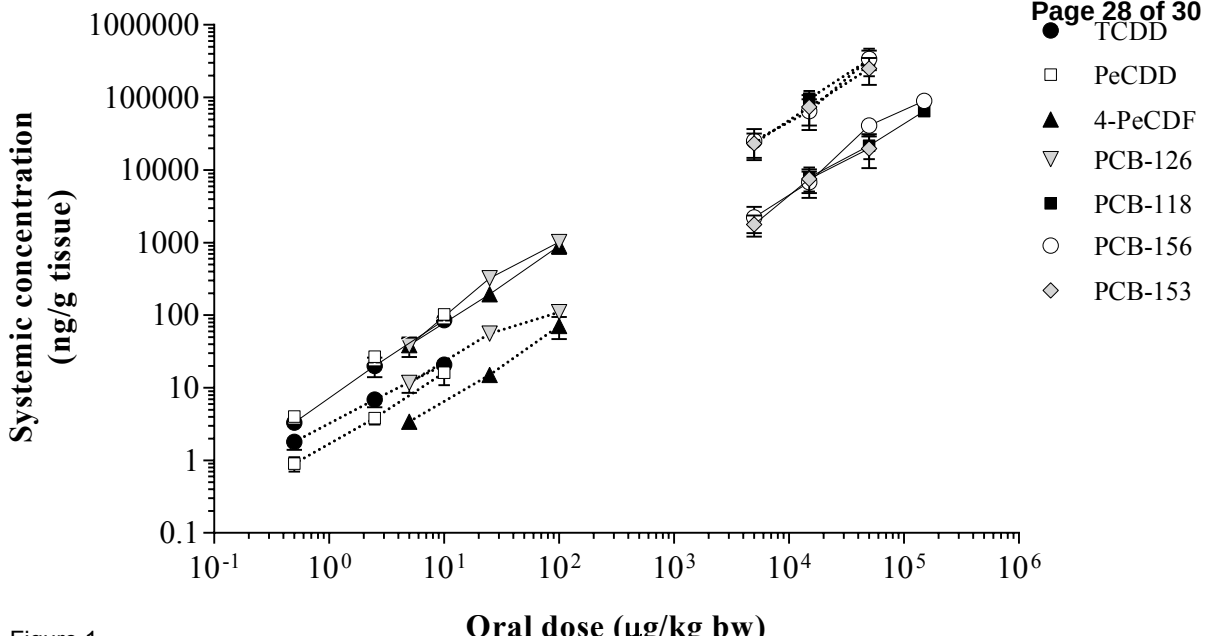
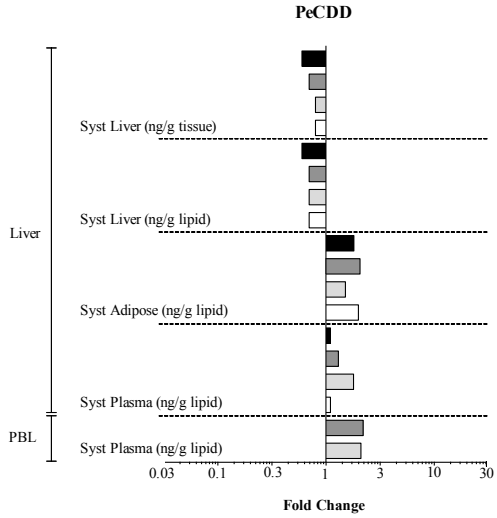
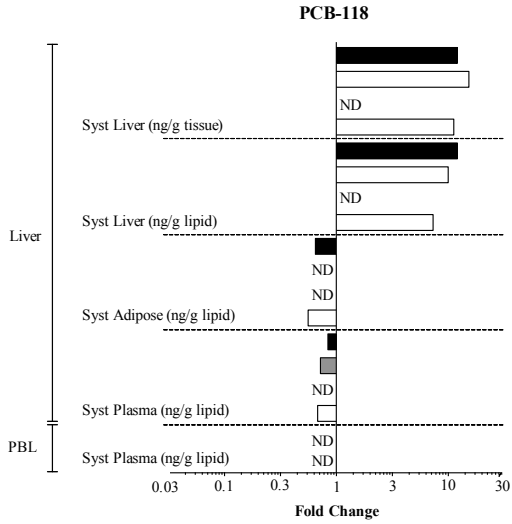
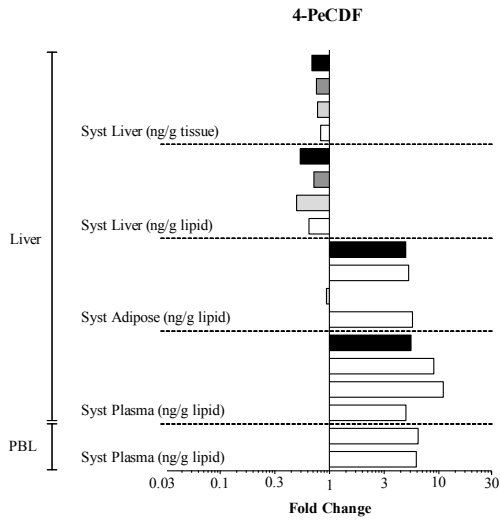

Systemic concentration (ng/g tissue)
1000000
100000
10000
1000
100
10
1
0.1
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
Oral dose (µg/kg bw)
TCDD
PeCDD
4-PeCDF
PCB-126
PCB-118
PCB-156
PCB-153

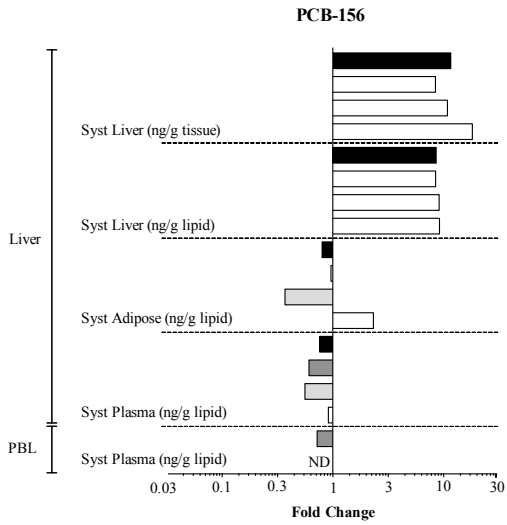
Figure 1

Figure 2


PeCDD
Liver
PBL
Syst Liver (ng/g tissue)
Syst Liver (ng/g lipid)
Syst Adipose (ng/g lipid)
Syst Plasma (ng/g lipid)
Syst Plasma (ng/g lipid)
0.03
0.1
0.3
1
3
10
30
Fold Change


PCB-118
Liver
PBL
ND
ND
ND
ND
ND
ND
ND
Syst Liver (ng/g tissue)
Syst Liver (ng/g lipid)
Syst Adipose (ng/g lipid)
Syst Plasma (ng/g lipid)
Syst Plasma (ng/g lipid)
0.03
0.1
0.3
1
3
10
30
Fold Change


4-PeCDF
Liver
PBL
Syst Liver (ng/g tissue)
Syst Liver (ng/g lipid)
Syst Adipose (ng/g lipid)
Syst Plasma (ng/g lipid)
Syst Plasma (ng/g lipid)
0.03
0.1
0.3
1
3
10
30
Fold Change


PCB-156
Liver
PBL
ND
Syst Liver (ng/g tissue)
Syst Liver (ng/g lipid)
Syst Adipose (ng/g lipid)
Syst Plasma (ng/g lipid)
Syst Plasma (ng/g lipid)
0.03
0.1
0.3
1
3
10
30
Fold Change

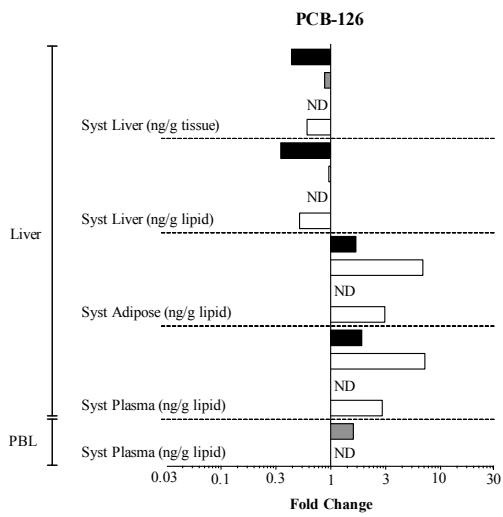
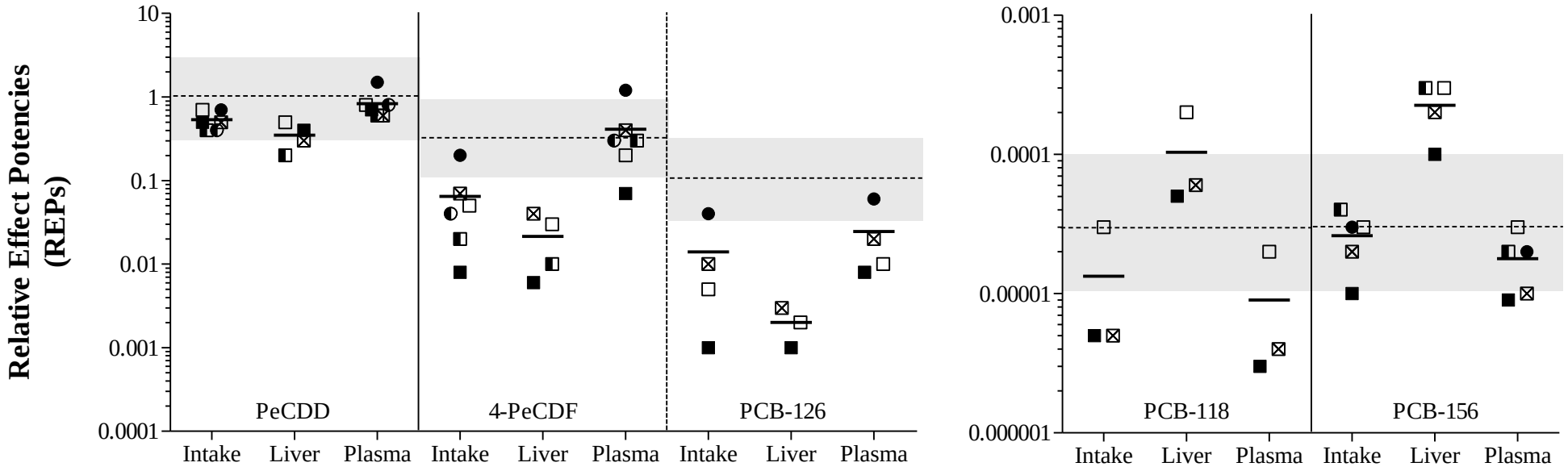

PCB-126
Liver
PBL
ND
ND
ND
ND
ND
Syst Liver (ng/g tissue)
Syst Liver (ng/g lipid)
Syst Adipose (ng/g lipid)
Syst Plasma (ng/g lipid)
Syst Plasma (ng/g lipid)
0.03
0.1
0.3
1
3
10
30
Fold Change

Figure 3:


Relative Effect Potencies (REPs)
10
1
0.1
0.01
0.001
0.0001
PeCDD
4-PeCDF
PCB-126
Intake
Liver
Plasma
0.001
0.0001
0.00001
0.000001
PCB-118
PCB-156